same as those aboveground, attracting not only parasitoids, but also hyperparasites and other food web members.

Symposium. Monday, 15:30. **8**

Root Zone Chemical Ecology; New Techniques for Below Ground Sampling and Analyses of Volatile Semiochemicals

Hans T. Alborn [1]; Fatma Kaplan[2]
[1] USDA ARS Center for Medical, Agricultural and Veterinary Entomology, Gainesville FL 32608; [2] Kaplan Schiller Research LLC and Biology Dept. University of Florida, Gainesville, FL 32611
Address for correspondence: hans.alborn@ars.usda.gov

The ban of the fumigant methyl bromide has led to a need for new methods to control soil-dwelling plant pests. The use of semiochemicals is one such avenue of research since studies of plants above ground release of volatile organic compounds (VOCs) in response to herbivory have resulted in effective control methods for insect pests and also plant roots might release induced VOCs that attract organisms such as entomopathogenic nematodes. However, studies of such below ground interactions lags because of the complexity of the system. For example, in addition to plants roots, potentially important VOCs can be produced also by microorganisms, insects and nematodes and in soil VOCs are released into a virtually static airspace where they disperse solely by diffusion. To bypass this complexity root-related VOCs have been sampled by transferring roots from a pot to an artificial environment where most of the air surrounding the roots is drawn through an adsorption filter that trap VOCs, or by maceration and solvent extraction. This creates an artificial VOC profile with little relevance to the system intended to be studied. To address the need for more sensitive and less intrusive *in vivo* studies of below-ground VOC governed interactions probes were designed for direct in-soil sampling. In combination with improved thermal desorption GC/MS analyses the probes allowed short sampling times and required removal of minimal air volumes. This technique makes it possible to continuously monitor and follow the dynamics of root zoon VOCs in response to insect or nematode infestations.

CONTRIBUTED PAPERS Monday, 14:00-16:00

BACTERIA 1

Contributed paper. Monday, 14:00. **9**

Discovery of Insecticidal Proteins from Non-Bacillus Bacterial Species

Nasser Yalpani[1]; Dan Altier[1], Jennifer Barry[1], Jarred Oral[2], Ute Schellenberger[2], Adane Negatu[1], Scott Diehn[1], Virginia Crane[1], Gary Sandahl[1], Joe Zhao[1], Dave Cerf[2], Claudia Perez Ortega[3], Mark Nelson[3], Analiza Alves[1], Lu Liu[2], Gusui Wu[1]
[1]DuPont Pioneer, Johnston, IA, U.S.A; [2]DuPont Pioneer, Hayward, CA, U.S.A.; [3] DuPont, Wilmington, DE, U.S.A.
Address for Correspondence: nasser.yalpani@pioneer.com

Crops expressing various *Bacillus thuringiensis*-derived insecticidal Cry protein genes have been on the market for over 15 years and have provided significant value to growers. Such products also provide a significant positive impact on the environment due to the reduced need for chemical insecticides. However, there remains the need for the discovery of alternative actives that can complement or substitute for Cry toxins. A screen of bacterial collections led to the discovery of several insecticidal protein genes with great potential for developing insect resistant crops. Two examples representing actives from non-Bacillus sources will be presented: PIP-1A is a 30 kD protein isolated form a *Pseudomonas* strain showing strong activity against hemipteran and certain lepidopteran pests. AfIP-1A and AfIP-1B is a pair of binary proteins isolated from an *Alcaligenes* strain demonstrating potent corn rootworm killing activity. Corn plants expressing this pair of proteins display high resistance to WCRW. Preliminary studies on AfIP-1A and AfIP-1B in terms of protein biochemical characteristics, insecticidal activity spectrum and insect mid-gut binding properties indicate this pair of binary proteins may function in ways similar to some Cry proteins from Bacillus sources. Our work demonstrates that bacteria that are not Bacillus can be valuable sources of insecticidal proteins.

Contributed paper. Monday, 14:15. **10**

Discovery and optimization of hemipteran-active proteins for Lygus control in cotton

James A. Baum, Waseem Akbar, Konasale Anilkumar, David Bowen, Robert S. Brown, Cathy Chay, Thomas Clark, Michael Pleau, Xiaohong Shi, Uma Sukuru, Moritz Von Rechenberg, Halong Vu, Brent Werner, Andrew Wollacott
Monsanto Company, 700 Chesterfield Parkway West, Chesterfield, Missouri 63017
Address for Correspondence: james.a.baum@monsanto.com

The plant bugs *Lygus hesperus* and *Lygus lineolaris* have emerged as economic pests of cotton in the United States. These hemipteran species are not controlled by the lepidopteran-specific insect control traits (*Bacillus thuringiensis* Cry proteins) found in genetically-modified commercial varieties of cotton. We have identified several novel Bt Cry proteins that are toxic to Lygus nymphs in artificial diet bioassays. Several of these proteins have been further modified to exhibit improved toxicity towards both Lygus species while retaining the insecticidal specificity of the parent protein. Cotton plants expressing modified Cry proteins show enhanced protection from Lygus feeding damage in the field.

Contributed paper. Monday, 14:30. **11**

Isolation and identification of potential biological control agent from *Tortrix viridana* L.(Lepidoptera: Tortricidae) pupae

Nurcan Albayrak Iskender[1]; Yaşar Aksu[2]
[1]Artvin Coruh University, Faculty of Arts and Sciences, Department of Biology, Artvin,Turkey; [2]Artvin Regional Forestry Management, Artvin, Turkey
Address for Correspondence: nurcaniskender25@hotmail.com

Tortrix viridana is one of the most important pest in the oak fields in Turkey. The aim of this study is to find a more effective and safe biological control agent against *Tortrix viridana.* For this purpose, pupae of *T. viridana* were collected from Artvin province, Turkey in 2013. According to the morphological, biochemical tests, API20E and API50CH panel test system and 16S rRNA gene sequence analysis, the bacterial isolates were identified as Serratia *liquefaciens* (Tv1), *Enterococcus* sp. (Tv2), *Rhodococcus erytropolis* (Tv3), *Rahnella aquatilis* (Tv4), *Curtobacterium flaccumfaciens* (Tv5), *Pseudomonas* sp. (Tv6). Future research will be tested insecticidal effects of these bacterial isolates against *T. viridana.*.

Contributed paper. Monday, 14:45. **12 STU**

Evolution of a Sensor Protein Controlling Production of an Insecticidal Toxin in Plant-Beneficial *Pseudomonas protegens*

Peter Kupferschmied [1], Maria Péchy-Tarr [1], Nicola Imperiali [1], Monika Maurhofer [2], Christoph Keel [1]
[1] Department of Fundamental Microbiology, University of Lausanne, Switzerland; [2] Plant Pathology, Institute of Integrative Biology, ETH Zurich, Switzerland
Address for Correspondence: peter.kupferschmied@unil.ch

Pseudomonas protegens is a plant-associated bacterium that is not only able to promote plant growth by efficiently protecting roots from attack by fungal phytopathogens but also can turn into an insect pathogen. The microorganism is capable of killing certain pest insects upon oral infection. The major goal of our work is to understand the molecular mechanisms that allow *P. protegens* and related bacteria to detect, to survive within and to kill the insect host. The entomopathogenic activity of *P. protegens* relies in part on the production of an insecticidal toxin termed Fit. We found that the pseudomonad produces the Fit toxin in the insect host, but not on plant roots, demonstrating that the bacterium is capable of distinguishing between these two environments. An array of sensor proteins makes bacteria able to sense the environment they live in and to adapt their behavior accordingly. Here we provide evidence that the sensor histidine kinase FitF is a key regulator of insecticidal toxin production. Our experimental data and bioinformatic analyses indicate that FitF shares a sensing domain with DctB, a histidine kinase regulating carbon uptake in Proteobacteria. This suggests that FitF has acquired its specificity through domain shuffling from a common ancestor. This particular event appeared to be crucial for host-dependent activation of toxin production and thus contributed to the evolution of insect pathogenicity in these bacteria. We propose that inhibition of the FitF sensor during root colonization is the underlying mechanism by which *P. protegens* differentiates between the plant and insect host..

Contributed paper. Monday, 15:00. **13 STU**

***Paenibacillus larvae*, the etiological agent of American Foulbrood, produces the catechol type siderophore bacillibactin**

Gillian Hertlein [1]; Sebastian Müller [2]; Eva Garcia-Gonzalez [1]; Roderich D. Süßmuth [2]; Elke Genersch [1,3]
[1] Institute for Bee Research Hohen Neuendorf, Friedrich-Engels-Str. 32, 16540 Hohen Neuendorf, Germany; [2]Technische Universität Berlin, Institut für Chemie, 10623 Berlin, Germany; [3]Freie Universität Berlin, Institute of Microbiology and Epizootics, Robert-von-Ostertag-Str. 7-13, 14163 Berlin, Germany
Address for correspondence: elke.genersch@rz.hu-berlin.de
Presenting author: gillian.hertlein@cms.hu-berlin.de

The Gram positive, spore forming bacteria *Paenibacillus larvae* is the causative agent of American Foulbrood, a fatal disease affecting the brood of honey bees. The whole vegetative life cycle of *P. larvae* takes place inside the larvae and all micronutrition must be obtained from the host- including iron- a scarce atom essential for growth of host and pathogen likewise. Bacteria often answer this iron deficiency with the production of siderophores, small molecules which act as powerful iron chelators. Such siderophores are often synthesized by mulitenzyme complexes through non-ribosomal peptide-synthetases (NRPS). The genes of these multienzyme complexes are arranged in giant gene clusters.
Here we present data on the identification of an NRPS gene cluster in *P. larvae* encoding the biosynthetic machinery for the production of a siderophore, which was identified as bacillibactin by MS/MS. Exposure bioassays with mutant *P. larvae* strains lacking bacillibactin production showed that neither total mortality nor disease progression in infected larvae was significantly changed compared to larvae infected with the corresponding wild-type strain. These results are in line with results published on the role of bacillibactin in other pathogenic bacteria like *Bacillus thuringiensis* and *B. anthracis*.

Contributed paper. Monday, 15:15. **14**

Two new *Bacillus thuringiensis* toxins active against Lepidoptera and Coleoptera.

Mikel Domínguez[1], Iñigo Ruiz de Escudero[1,2], Isabel Matas[2], Leopoldo Palma[1,2], Delia Muñoz[2], Primitivo Caballero[1,2]
[1]Bioinsecticidas Microbianos, Instituto de Agrobiotecnología, Mutilva, Spain.[2]Laboratorio de Entomología Agrícola y Patología de Insectos, Universidad Pública de Navarra, Pamplona, Spain
Address for Correspondence: iruiz@unavarra.es

The entomopathogenic spore-forming bacteria, *Bacillus thuringiensis* (Bt), is widely distributed around the world and is able to produce toxins with insecticidal activity during the vegetative and sporulation phase. The great genetic variety of *B. thuringiensis* strains represents a huge diversity of potential insecticidal proteins. The host range of these proteins is highly variable, but includes a large number of species of the most damaging lepidopteran insect pests and also, other harmful species of the orders Diptera, Coleoptera and Hymenoptera. In order to extend the number of Bt proteins active against important coleopteran and lepidopteran pests, total DNA of a strain from a Spanish collection was completely sequenced. Two ORFs of ~900 bp were selected due to their low identity with other Bt proteins and were cloned in a Bt expression plasmid. Proteins were produced and their insecticidal activity was determined. Bm_47 protein was toxic against *Leptinotarsa decemlineata*, with an LC_{50} of 54 µg/ml, while Bm_1711 protein was active against the lepidopterans *Helicoverpa armigera* and *Ostrinia nubilalis*, with an LC_{50} of 164 and 34 ng/cm^2, respectively. We discuss the importance of this protein to combat species of coleopteran and lepidopteran pests, including species that have developed resistance to other Bt toxins..

Contributed paper. Monday, 15:30. **15-STU**

Entomopathogenic *Bacillus thuringiensis* as PGPR

Jiaheling Qi[1,2]; Daigo Aiuchi[2]; Shin-ichiro Asano[3]; Masanori Koike[2]
[1]The United Graduate School of Agricultural Sciences, Iwate University, Iwate Japan; [2] Department of Agro-environmental Science, Obihiro University of Agriculture & Veterinary Medicine, Obihiro 080-8555, Japan.;[3] Department of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, N9 W9, Sapporo 060-8589, Japan
Address for Correspondence: koike@obihiro.ac.jp

Bacillus thuringiensis has been used as an effective bio-insecticide because it produces the proteins Cry and Cyt, which are highly toxic to insects in certain situations. But, recently *B. thuringiensis* was treated as a biological control agent which could control the plant disease. We already demonstrated that the antagonistic activity of *B. thuringiensis AS17 japonensis, AS18 kurstaki* against *Fusarium oxysporum* f.sp. *lycospersici* race2 (FOL) was examined by dual culture technique(Qi et al. 2013). In this study, *B. thuringiensis* strains could control the development of wilt symptoms caused by FOL in tomato plants was confirmed. Inoculate six

strains of *B. thuringiensis* suspension to the tomato seedlings in pot, and transplanted the treated tomato seedlings to FOL infested soil, after 4 weeks the development of wilt symptoms and wilting score become less than control, especially *B. thuringiensis AS17 japonensis* and *AS20 CR371-H*. Also, this study proved that *B. thuringiensis* strains are PGPR. PGPR (Plant growth promoting rhizobacteria) are beneficial bacteria which have the ability to colonize the plant roots and either promotes plant growth through direct action or via biological control of plant diseases. Six strain of Insect Pathogenic *Bacillus thuringiensis* were tested for PGPR effect. Culture filtrates of six strains had remarkable plant growth promotion activity in tomato and alfalfa plants; in each plant after treatment of culture filtrates, both of seed germination rates and the fresh weight were increased compared with control treatment.

Contributed paper. Monday, 15:45. **16**

Vibrios pathogenic for oysters are found associated to plankton species. What possible consequences on pathogen transmission to oysters?

Carmen Lopez-Joven[1], Jean-Luc Rolland [1]*, Eric Abaddie [2], Mohamed Laabir [1], Estelle Masseret [1], Audrey Vanhove [1], Audrey Caro [1], Delphine Bonnet [1], Delphine Destoumieux-Garzón [1]

[1]Ecology of coastal marine systems, UMR 5119, CNRS, Ifremer, IRD, University of Montpellier, France; [2]Laboratoire Environnement Ressource du Languedoc Roussillon, Ifremer, Sète, France.

* Address for Correspondence: jlrollan@ifremer.fr

Vibrios cause major losses in shellfish farming and are associated to recurrent mortalities of oysters. However, to date, the role of plankton species in the transmission of pathogenic vibrios in oyster *Crassostrea gigas* is largely unknown. The main objective of the present study was to identify *in situ* and *in vitro* the interactions of pathogenic Vibrios with local species of planktons from different sites of Thau lagoon, an important region for shellfish farming in south of France. Quantitative-PCR was used to monitor *Vibrio splendidus* and *Vibrio aestuarianus* over the year 2013 at two sites of the Thau lagoon. Out of the oyster farm area, *V. splendidus* was found from May to July and from June to August associated to 5-180 µm and >180µm plankton fractions, respectively. *V. aestuarianus* was also detected in fraction 5-180 µm in May and >180µm in August, before and after the warmer months of the year. For the farm oysters point, *V. splendidus* was found in January and June associated with the 5-180 µm plankton and with the >180 µm fraction in spring and winter. *V. aestuarianus* was not detected. In laboratory controlled conditions, by using a GFP-expressing *V. splendidus* LGP32 and epifluorescence microscopy, we showed that *V. splendidus* LGP32 exhibits strong interactions with copepods of the *Acartia* and *Paracartia* genius as well as with microalgae of the *Alexandrium* genius. Altogether, our data show that vibrios pathogenic for oysters can establish close associations with plankton species, which may enhance the transmission of pathogenic vibrios to oysters.

VIRUSES 1

Contributed paper. Monday, 14:00. **17**

Investigation of Baculovirus RNA Polymerase Subunit Protein-Protein Interactions with *in vivo* Bimolecular Fluorescence Complementation Assays

Jessica Breznik, Nicola Johnson, Mustapha El-Ayoubi and Eric B Carstens

Queen's University, Kingston, Canada

Address for Correspondence: Carstens@queensu.ca

Baculovirus transcription utilizes two different DNA-directed RNA polymerases (RNAPs): the insect host RNAP transcribes early genes while a virus RNAP transcribes late and very late genes. The virus RNAP consists of four proteins: P47, LEF-4, LEF-8 and LEF- 9. Conserved motifs in LEF-8 and LEF-9 suggest that the interface of these subunits forms the catalytic site of the RNAP, while LEF-4 has RNA capping-associated enzymatic activities. No specific function has yet been demonstrated for P47. To investigate the *in vivo* intracellular localization and interactions of these proteins, two individually non-fluorescent fragments (V1 and V2) of the Venus yellow fluorescent protein were fused with the N-termini of each RNAP subunit in plasmid expression vectors. We also constructed similar fusions with two components of the virus replisome complex, LEF-3 and P143, and of the host *Spodoptera frugiperda* TATA binding protein. Bacmids, expressing each of these fusion proteins, were constructed and used to generate recombinant viruses expressing each of the V1- or V2-tagged protein subunits. Protein-protein interactions of these subunits were investigated using bimolecular fluorescence complementation assays. Co-infections were used to investigate the interactions of these subunits in the presence of the full complement of virus proteins. Reciprocal co-transfections of the original plasmid constructs were performed to investigate the potential for these proteins to form homo-oligomers, as well as their ability to interact with heterologous partners in the absence of any other viral proteins. The results of co-transfection and co-infection assays will be presented.

Contributed paper. Monday, 14:15. **18-STU**

Characterization and Quantitative Analysis of *Autographa californica* Multiple Nucleopolyhedrovirus (AcMNPV) FP25K Localization and Aggregate Formation During Cell Infection

Tyler A. Garretson and Xiao-Wen Cheng

Department of Microbiology, 32 Pearson Hall, Miami University, Oxford, Ohio 45056 USA

Address for Correspondence: garretta@miamioh.edu

Localization of AcMNPV FP25K was previously studied by western blot using fractionation. This study, however, was not quantitative. By inactivating the endogenous *fp25k* gene through passage of the AcBacmid in Sf21 cells and subsequent insertion of an *fp25k-egfp* fusion gene at the *polyhedrin* locus, we investigated FP25K localization during infection. Western blot confirmed the 53-kDa FP25K-EGFP fusion protein from infected cells. By using a nuclear stain, we were able to assess and quantify the nuclear to cytoplasmic localization of FP25K-EGFP during Hi5 and Sf9 cell infection through confocal microscopy. During late phase of infection, small aggregates were formed and FP25K-EGFP was found exclusively in the cytoplasm. However, during very late phase of infection, larger aggregates were observed in both the